

Attorney Docket No. P66602US0
Application No. 09/841,157

Amendments to the specification:

Replace paragraph [0021] (page 3, lines 22-26) with the following amended paragraph:

[0021] FIG. 6a illustrates an electrophoresis pattern in case cy3-pfM12 (5' cy3-AGA ACG CGC CTG 3') (SEQ ID NO: 4) which was obtained in Example 2 was used as a primer, and FIG. 6b illustrates an electrophoresis pattern in case FITC/LCS (5' FITC-CA GGA AAC AGC TAT GAC 3') (SEQ ID NO: 38) was used as a primer.

Replace paragraph [0030] (page 6, line 22, to page 8, line 6) with the following amended paragraph:

[0030] Apart from the normal PCR method, an appropriate sequence, length and kind of a primer can be appropriately selected for the random PCR. Experimentally and analytically the most simple system, however, is the case where a relatively short single primer is used. Even in the case a single primer is used, and a genome which is derived from the same microorganism is amplified, a kind and quantity of a double-stranded DNA fragment which can be amplified depend on the sequence and length of the primer. In addition even in the case a single primer and a primer which has the same sequence and length are used, a kind and quantity of a double-stranded DNA fragment which can be amplified depend on the microorganism from which the genome is derived. Considering these facts, sequence, length, and kind of a primer are appropriately selected. Too long primer can have the hair-pin structure, can

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prevent annealing, and tends to lower a yield of a PCR product. Too short primer does not adequately stabilize a bonding containing mismatch (es), and tends to lower a yield of a PCR product. A length (base length) of a primer is suitably, for example, 8-20, preferably 10-16, more preferably 10-12, and most preferably 12. An oligonucleotide which has a length of 12 bases can be used as a primer. Such oligonucleotide primers include pM4 (dCAGTCAGGACGT) (SEQ ID NO: 3), pM12 (dAGAACGCGCCTG) (SEQ ID NO: 4), pM14 (dCGTCGCTAATTAA) (SEQ ID NO: 5), pM19 (dCAGGGCGCGTAC) (SEQ ID NO: 6), (dA)₁₂ (dAAAAAAAAAAAAA) (SEQ ID NO: 7), (dA₃T₃)₂ (dAAATTTAAATTT) (SEQ ID NO: 8), (dAATT)₃ (dAATTAATTAATT) (SEQ ID NO: 9), (dACG)₄ (dACGACGACGACG) (SEQ ID NO: 10), (dAT)₆ (dATATATATATAT) (SEQ ID NO: 11), (dC)₁₂ (dCCCCCCCCCCCC) (SEQ ID NO: 12), (dCCGG)₃ (dCCGGCCGGCCGG) (SEQ ID NO: 13), (dG)₁₂ (dGGGGGGGGGGGGGG) (SEQ ID NO: 15), (dGA)₆ (dGAGAGAGAGAGA) (SEQ ID NO: 16), (dGGCC)₃ (dGGCCGGCCGGCC) (SEQ ID NO: 17), (dCT)₆ (dCTCTCTCTCTCT) (SEQ ID NO: 14), (dT)₁₂ (dTTTTTTTTTTTTTT) (SEQ ID NO: 18), (dT₃G₃)₂ (dTTTGGGTTTGGG) (SEQ ID NO: 19), (dTGC)₄ (dTGCTGCTGCTGC) (SEQ ID NO: 20), (TA)₄C₂AC (dTATATATACCAC) (SEQ ID NO: 21), Cohesive1 (dGGGCGGCGACCT) (SEQ ID NO: 22), Cohesive2 (dAGGTCGCCGCCC) (SEQ ID NO: 23), G4sand (dGGGGTCGAGGGG) (SEQ ID NO: 24), GCTA₉

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(dGCTAAAAAAAAAA) (SEQ ID NO: 25), notG (dCAATTCTACAAC) (SEQ ID NO: 26), notT (dACGAGCGAGCGC) (SEQ ID NO: 27), Promote1 (dTATAATTATAAT) (SEQ ID NO: 28), Promote2 (dATTATAATTATA) (SEQ ID NO: 29), SD1 (dGATCACCTCCTTA) (SEQ ID NO: 30), SD2 (dTAAGGAGGTGATC) (SEQ ID NO: 31), Telomere1 (dCCCACCCA(CCCA) (SEQ ID NO: 32), Telomere2 (dTGGGTGGGTGGG) (SEQ ID NO: 33), FITC17-H-3' (5' GAGGAAACAGCTATGAGATCT TCTC 3') (SEQ ID NO: 34), FITC17-H-5' (5' CAG GAA ACA GCT ATG ACG TTC TCA C 3') (SEQ ID NO: 35), LH-7-3' (5' GGC GAT ATC CCT GAA A 3') (SEQ ID NO: 36), LH-7-5' (5' TAT TAT TTC CGC AAA G 3') (SEQ ID NO: 37), M13 Reverse (5' CAG GAA ACA GCT ATG AC 3') (SEQ ID NO: 38), cy3-pfM12 (5' cy3-AGA ACG CGC CTG 3') (SEQ ID NO: 39) (with cy3 fluorescence), FITC/UCS (5' FITC-CA GGA AAC AGC TAT GAC 3') (SEQ ID NO: 40) (with FITC fluorescence), MA1-FITC (5' FITC-TGCTAC GTC TCT TCC GAT GCT GTC TTT CGC T 3') (SEQ ID NO: 41) (with FITC fluorescence), cy3-MA1 (5' cy3-TGC TAC GTC TCT TCC GAT GCT GTC TTT CGC T 3') (SEQ ID NO: 42) (with cy3 fluorescence), HEX-pfM11 (5' HEX-GAA CCT CCC GAC 3') (SEQ ID NO: 43) (with Hex fluorescence), TAM-TGC4 (5' TAM-TGC TGC TGC TGC 3') (SEQ ID NO: 44) (with Tamara fluorescence), and the like.

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Replace paragraph [0102] (paragraph bridging pages 24 and 25) with the following amended paragraph:

[0102] One hundred μ l of reaction mixture which contains 200 μ M dNTP (N = G, A, T, C), 0.5 μ M primer (pfM12 (dAGAACGCGCCTG) (SEQ ID NO: 39)), 10 mM Tris-HCl (pH 9.0), 50 mM KCl, 2.5 mM $MgCl_2$, 0.1% Triton X-100, 0.02 unit/ μ l Taq DNA polymerase, and an appropriate amount of template DNA (3 μ l of a DNA solution was added, and the volume of the reaction mixture was adjusted to 100 μ l) was prepared for each DNA. Double-stranded DNA fragments were prepared by PCR comprising the steps of 1) the treatment at 94 °C for 1 min, 2) 20-30 times repeating the cycle comprising denaturation at 94 °C. for 30 min, annealing at 28 °C. for 2 min, and elongation at 47 °C. for 2 min, followed by 3) the treatment at 47 °C. for 2 min.

Replace paragraph [0116] (page 27, lines 7-23) with the following amended paragraph:

[0116] Random PCR was carried out under the same condition except using DNA of *Bacillus subtilis* as a template, and cy3-pfM12 (5' cy3-AGA ACG CGC (TG 3') (SEQ ID NO: 4) which contains phosphor cy3 or FITC/UCS (5' FITC-CA GGA AAC AGC TAT GAC 3') (5' FITC-CAGGAAACAG CTATGAC 3') (SEQ ID NO: 40) which contains phosphor FITC as a primer for each primer, and the obtained double-stranded amplified DNA fragments were mixed, and TGGE was carried out in a way which is similar to Example 1. An electrophoresis pattern by TGGE was

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visualized with an excitation/fluorescent wavelength of 550 nm/570 nm (phosphor cy3) or 494 nm/519 nm (phosphor FITC). FIG. 6a illustrates an electrophoresis pattern in the case where cy3-pfM12 (5'-cy3-AGA ACG CGC CTG 3') (SEQ ID NO: 4) was used as a primer, and FIG. 6b illustrates an electrophoresis pattern in the case where FITC/UCS (~~5'-FITC-CA GCA AAC AGC TAT GAC -3'~~) (5'-FITC-CAGGAAACAG CTATGAC 3') (SEQ ID NO: 40) was used as a primer. Identification dots were extracted from each pattern. The microorganism which was used as a template was identified as *Bacillus subtilis* based on the obtained identification dots.